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Urine-Based *Trichomonas vaginalis* PCR Detection Kit — Product Insert

Product # 52000

Pathogen Information

Trichomonas vaginalis is a protozoan pathogen of the human urogenital tract. A substantial proportion of infections are asymptomatic, necessitating reliable testing methods. Trichomoniasis is also implicated in various other genito-urinary syndromes, including cervicitis, epididymitis and prostatitis. Diagnosis of trichomoniasis based solely on clinical signs and symptoms is unreliable because the spectrum of infection is broad and other sexually transmitted pathogens can cause similar signs and symptoms. Diagnosis is particularly challenging in men, where infections are characterized by fewer organisms than infections in women. Early rapid diagnosis of this disease is essential to treat the illness before any serious complications arise.

Principle of the Test

Norgen's Urine-Based *Trichomonas vaginalis* PCR Detection Kit constituents a ready-to-use system for the isolation and detection of *Trichomonas vaginalis* using end-point PCR. The kit first allows for the isolation of *T. vaginalis* DNA from urine samples using spin-column chromatography based on Norgen's proprietary resin. The *T. vaginalis* DNA is isolated free from inhibitors, and can then be used as the template in a PCR reaction for *T. vaginalis* detection using the provided *T. vaginalis* Master Mix. The *T. vaginalis* Mastermix contains reagents and enzymes for the specific amplification of a 335 region of the genome. In addition, Norgen's *T. vaginalis* PCR Detection contains a second Mastermix, the Control 2x PCR Master Mix, which can be used to identify possible PCR inhibition and/or inadequate isolation via a separate PCR reaction with the use of the provided *Isolation Control (IsoC)*. This kit is designed to allow for the testing of 24 samples.

Kit Components:

Component	Contents
Solution A	10 mL
Solution B	15 mL
Wash Solution	9 mL
Elution Buffer	3 mL
Mini Filter Spin Columns	24
Collection Tubes	24
Elution tubes (1.7 mL)	24
TRI 2x PCR Master Mix	0.35 mL
Control 2x PCR Master Mix	0.35 mL
Isolation Control (IsoC)*[a]	0.3 mL
TRI Positive Control (PosC)*[b]	0.1 mL
Nuclease Free-Water	1.25 mL
Norgen's DNA Marker	0.1 mL
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** IsoC = Isolation Control ; PosC= Positive Control*
[a] The isolation control is a cloned PCR product.
[b] The positive control is gnomic DNA of Trichomonas vaginalis

Customer-Supplied Reagents and Equipment

- Disposable powder-free gloves
- Centrifuge with a swinging bucket rotor capable of 2000 RPM
- Benchtop microcentrifuge
- Micropipettors
- Sterile pipette tips with filters
- PCR tubes
- Lysozyme
- 96 – 100% ethanol
- 60°C incubator
- 15 mL tubes

Storage Conditions and Product Stability

All buffers should be kept tightly sealed and stored at room temperature (15-25°C) for up to 1 year without showing any reduction in performance.

The TRI 2x PCR Master Mix, Control 2x PCR Master Mix, Isolation Control (IsoC), the TRI Positive Control (PosC) and the Nuclease-Free Water should be kept tightly sealed and stored at -20°C for up to 1 year without showing any reduction in performance. Repeated thawing and freezing (> 2 x) should be avoided, as this may reduce the sensitivity. If the reagents are to be used only intermittently, they should be frozen in aliquots.

General Precautions

The user should exercise the following precautions when using the kit:

- Use sterile pipette tips with filters.
- Store and extract positive material (specimens, controls and amplicons) separately from all other reagents and add it to the reaction mix in a spatially separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Work quickly on ice.

Quality Control

In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's Urine-Based *Trichomonas vaginalis* PCR Detection Kit, including the TRI 2x PCR Master Mix, Control 2x PCR Master Mix, Isolation Control and TRI Positive Control are tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

Norgen's Urine-Based *Trichomonas vaginalis* PCR Detection Kit is designed for research purposes only.

Product Warranty and Satisfaction Guarantee

NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use.

Disclaimers

Solution B contains guanidine hydrochloride, and should be handled with care. Guanidine hydrochloride forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions. If liquid containing these solutions is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Safety Information
Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

1. Protocol

A. Specimen Collection, Storage and Transport

General Precautions

- Follow universal precautions. All patient specimens should be considered as potentially infectious and handled accordingly.
- Wear personal protective equipment, including gloves and lab coats when handling kit reagents.
- Wash hands thoroughly when finished performing the test.
- Do not smoke, drink or eat in areas where kit reagents and/or human specimens are being used.
- Dispose of unused kit reagents and human specimens according to local, provincial or federal regulations.
- Do not use supplies and equipment across the dedicated areas of specimen extraction and sample preparation. No cross-movement should be allowed between the different areas.
- Personal protective equipment, such as laboratory coats and disposable gloves, should be area specific.
- As contamination of patient specimens or reagents can produce erroneous results, it is essential to use aseptic techniques.
- Pipette and handle reagents carefully to avoid mixing of the samples.
- Use proper pipetting techniques and maintain the same pipetting pattern throughout the procedure to ensure optimal and reproducible values.
- Do not substitute or mix reagents from different kit lots or from other manufacturers

1. Specimen Collection and Sample Storage

- Midstream urine samples should be collected, as the first flow of urine has been shown to have a higher rate of contamination (Morimoto *et al*., 2003).
- It is highly recommended that urine samples be collected using Norgen's Urine Collection and Preservation Tubes (Cat# 18111). The urine samples can be stored for at least one year at room temperature when collected directly using Norgen's Urine Collection and Preservation Tubes.
- Alternatively, urine samples collected using any other collection and preservation systems or reagents are also compatible with this kit.

2. Sample Transport

- Sample material should be transported in a shatterproof, leak-proof transport container as a matter of principle. Thus, a potential danger of infection due to a leakage of sample can be avoided.
- The samples should be transported following the local and national instructions for the transport of pathogen material.

B. Isolation of DNA from Urine

Notes:

- Do not spin down or filter the urine sample before proceeding with the isolation, as this could negatively affect the isolation of Chlamydia DNA.
- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- Preheat an incubator or heating block to 60°C.
- Prepare a working concentration of **Wash Solution** by adding 21 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution**. This will give you a final volume of 30 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Prepare a 400 mg/mL stock solution (approximately 1.7 $x10^7$ units/mL) of lysozyme as per supplier's instructions.
- **Isolation Control (IsoC)**
    - An Isolation Control (IsoC) is supplied. This allows the user to control the DNA isolation procedure. For this assay, add the Isolation Control (IsoC) as indicated during the isolation procedure
    - The Isolation Control (IsoC) must not be added to the sample material directly.
    - Do not freeze and thaw the Isolation Control (IsoC) more than 2 times.
    - The Isolation Control (IsoC) must be kept on ice at all times during the isolation procedure.
- The PCR components of the Urine-Based Chlamydia PCR Detection Kit should remain at -20°C until DNA is extracted and ready for PCR amplification.
- It is important to work quickly during this procedure.

1. Add 300 µL of **Solution A** to 10 mL urine sample. Mix well by vortexing for 10 seconds. **(Note 1: Solution A must be mixed well before every pipetting**)

2. Centrifuge for **5 minutes at 2,000 RPM**, then discard the supernatant carefully in order not to dislodge the precipitated slurry pellet..

3. Add 20 µL of the previously prepared **lysozyme** to the precipitated slurry pellet. **Vortex for 10 seconds**. Incubate the mixture at **60°C for 20 minutes**

4. Add 500 µL **Solution B** to the precipitated slurry pellet, mix well by vortexing for 10 seconds.

5. Add 10 µL **Isolation Control (IsoC)** to the mixture from **Step 4.**

6. Add 500 µL of **96-100% Ethanol** to the mix from **Step 4**, mix well by vortexing for 10 seconds.

7. Transfer 650 µL from the previous mix into a Mini Filter Spin column and centrifuge for **1 minute** at 14,000 RPM. Discard the flowthrough and reassemble the spin column with its collection tube.

8. Repeat **Step 7** until the entire mixture from **Step 6** has been transferred to the Mini Filter Spin Column.

9. Apply 400 µL of **Wash Solution** to the column and centrifuge for **1 minute**. Discard the flowthrough and reassemble the spin column with its collection tube.

10. Repeat Step 9 to wash column second time.

11. Wash the column a third time by adding another 400 µL of **Wash Solution** to the column and centrifuge for **1 minute**. Discard the flow-through and reassemble the spin column with its collection tube.

12. Spin the column for **2 minutes** empty at 14,000 RPM in order to thoroughly dry the resin. Discard the collection tube.

13. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply 100 µL of **Elution Buffer** to the column and centrifuge for **2 minutes at 2,000 RPM,** followed by **1 minute at 14,000 RPM**.

C. *Trichomonas* PCR Assay Preparation

Notes:

- Before use, suitable amounts of all PCR components should be completely thawed at room temperature, vortexed and centrifuged briefly.
- The amount of TRI 2x PCR Master Mix and Control PCR Master Mix provided is enough for up to 32 PCR reactions (24 sample PCR, 4 positive control PCR and 4 no template control PCR).
- For each sample, one PCR reaction using the **TRI *2x* PCR Mastermix** and one PCR reaction using **Control 2x PCR Mastermix** should be set up in order to have a proper interpretation of the result.
- For every PCR run, one reaction containing *TRI* Positive Control (*PosC*) and one reaction as no template control must be included for proper interpretation of results.
- The recommended minimum number of DNA samples tested per PCR run is 6.
- Using a lower volume from the sample than recommended may affect the sensitivity of *Trichomonas vaginalis* Limit of Detection.

1. Prepare the PCR for sample detection (Set #1, using **TRI 2x PCR Mastermix**) and control detection (Set #2, using **Control 2x PCR Mastermix**) as shown in Table 1 below. The recommended amount of sample DNA to be used is 2.5 µL. However, a volume between 1 and 5 µL of sample DNA may be used as template. Ensure that one TRI detection reaction and one control reaction is prepared for each DNA sample. Adjust the final volume of the PCR reaction to 20 µL using the Nuclease-Free Water provided.

Table 1. PCR Assay Preparation

PCR Components	Volume Per PCR Reaction
TRI 2x PCR Master Mix Or Control 2x PCR Master Mix	**10 µL**
Sample DNA	**2.5 µL**
Nuclease-Free Water	**7.5 µL**
Total Volume	**20 µL**

2. For every PCR set, prepare **one** positive control PCR as shown in Table 2 below:

Table 2. PCR Positive Control Preparation

PCR Components	Volume Per PCR Reaction
TRI 2x PCR Master Mix Or Control 2x PCR Master Mix	**10 µL**
TRI *Positive Control (PosC)*	**10 µL**
Total Volume	**20 µL**

3. For every PCR set, prepare **one** no template control PCR as shown in Table 3 below:

Table 3. PCR Negative Control Preparation

PCR Components	Volume Per PCR Reaction
TRI 2x PCR Master Mix Or Control 2x PCR Master Mix	**10 µL**
Nuclease-Free Water	**10 µL**
Total Volume	**20 µL**

D. *Trichomonas* PCR Assay Programming

1. Program the thermocylcer according to the program shown in Table 4 below.
2. Run one step PCR.

Table 4. *Trichomonas* PCR Assay Program

PCR Cycle	Step	Temperature	Duration
Cycle 1	**Step 1**	**95°C**	**3 min**
Cycle 2 (35x)	**Step 1**	**94°C**	**15 sec**
	Step 2	**52°C**	**15 sec**
	Step 3	**72°C**	**30 sec**
Cycle 3	**Step 1**	**72°C**	**5 min**
Cycle 4	**Step 1**	**4°C**	**∞**

E. *Trichomonas* PCR Assay Results Interpretation

1. For the analysis of the PCR data, the entire 20 µL PCR Reaction should be loaded on a 1X TAE 1.5% Agarose DNA gel along with 10 µL of Norgen's DNA Marker (provided).
2. The PCR products should be resolved on the 1X TAE 1.5% Agarose gel at 150V for 30 minutes (Gel running time will be vary depending on an electrophoresis apparatus).
3. Sample results are provided below:

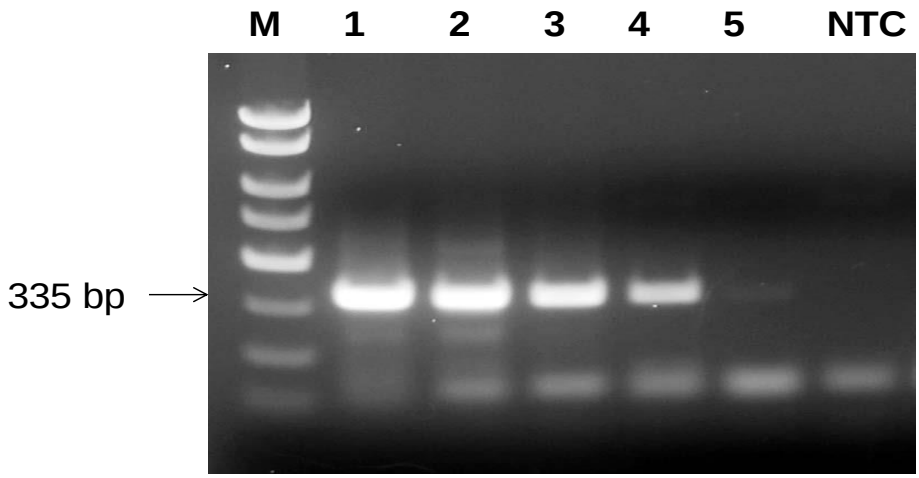

Figure 1: A representative 1X TAE 1.5% agarose gel showing the amplification of *Trichomonas* at different concentrations (*Trichomonas* Target). The size of the *Trichomonas* target amplicon corresponds to 335 bp as represented by the provided DNA Marker (M). **NTC** = Negative Control.

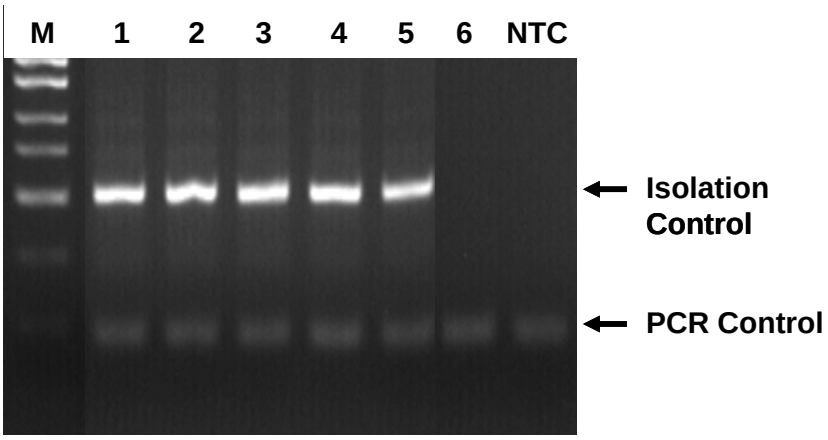

Figure 2: A representative 1X TAE 1.5% agarose gel showing the amplification of **Isolation Control** and **PCR Control** under different conditions using the **Control 2x PCR Mastermix**. The size of the Isolation Control amplicon and PCR Control amplicon correspond to 499 bp and 150 bp, respectively, as represented by the provided DNA Marker (M). Lanes 1 to 5 showed detection of both Isolation Control and PCR Control, suggesting that the DNA isolation as well as the PCR reaction was successful. Lane 6 showed only the detection of PCR Control suggesting that while the PCR was successful, the isolation failed to recover even the spiked-in Isolation control. **NTC** = Negative Control.

Table 5. Interpretation of PCR Assay Results

Input Type	Target reaction	Control Reaction		Interpretation
	***Trichomonas* Target Band (335 bp)**	***IsoC* Band (499 bp)**	***PCRC* Band (150 bp)**	
Positive Control	X	X	X	Valid
Negative Control			X	Valid
Sample	X	X	X	Positive
Sample		X	X	Negative
Sample			X	Re-test
Sample				Re-test
Sample		X		Negative
Sample	X		X	Positive
Sample	X	X		Positive
Sample	X			Re-test

For results obtained that are not covered in Table 5 above, please refer to the Troubleshooting Section.

****** Ignore any bands that appears in-between the Isolation Control Band and the PCR Control Band.

F. Specificity

- The specificity of Norgen's Urine-Based *Trichomonas vaginalis* PCR Detection Kit is first and foremost ensured by the selection of the *Trichomonas* specific primers, as well as the selection of stringent reaction conditions. The *Trichomonas* specific primers were checked for possible homologies to GenBank published sequences by sequence comparison analysis and published *Trichomonas* strains.

G. Linear Range

- The linear range of Norgen's Urine-Based *Trichomonas vaginalis* PCR Detection Kit was determined by analysing a dilution series of a *Trichomonas* quantification standards ranging from 1pg to 10 ng.
- Each dilution has been tested in replicates (n = 4) using Norgen's Urine-Based *Trichomonas vaginalis* PCR Detection Kit on a 1X TAE 1.5% agarose gel.
- The linear range of Norgen's Urine-Based *Trichomonas vaginalis* PCR Detection Kit has been determined to cover concentrations from 6 copies/µL to at least 6 x 10^5 copies/µL
- Under the conditions of the Norgen's Urine-Based *Trichomonas* DNA Isolation procedure, Norgen's *Trichomonas vaginalis* PCR Detection Kit covers a linear range from 100 copies/mL urine to at least 1 x 10^6 copies/mL urine

Frequently Asked Questions

1. **How many samples should be included per PCR run?**
   - Norgen's Urine-Based *Trichomonas vaginalis* PCR Detection Kit is designed to test 24 samples. For every 6 samples, a non-template control (Nuclease Free Water) and a Positive Control must be included. It is preferable to pool and test 6 samples at a time. If not, the provided Positive Control is enough to run 3 samples at a time.

2. **How can I interpret my results if neither the PCR control nor the Isolation Control (*IsoC*) amplifies?**
   - If neither the PCR control nor the Isolation Control (*IsoC*) amplifies, the sample must be re-tested. If the positive control showed amplification, then the problem occurred during the isolation, where as if the Positive control did not amplify, therefore the problem has occurred during the setup of the PCR assay reaction.

3. **How should it be interpreted if only the PCR control showed amplification but neither the *Trichomonas* target nor the Isolation control amplified for a sample?**
   - This indicates a poor isolation. The isolation procedure must be repeated.

4. **How should it be interpreted if only the Isolation Control (*IsoC*) was amplified in a sample?**
   - The sample tested can be considered as ***Trichomonas*** negative.

5. **How should it be interpreted if the PCR control and the *Trichomonas* target showed amplification in a sample?**
   - The sample tested can be considered positive. It could happen when too much template was added to the reaction.

6. **How should it be interpreted if only the *Trichomonas* target and the PCR control were amplified in a sample?**
   - The sample tested can be considered as ***Trichomonas*** positive.

7. **How should it be interpreted if only the *Trichomonas* target was amplified in a sample?**
   - It is recommended that the isolation is repeated.

8. **How should it be interpreted if only the PCR control and the Isolation control showed amplification in a sample?**
   - The sample tested can be considered negative

9. **What if I forgot to do a dry spin after my third wash?**
   - Your first DNA elution will be contaminated with the Wash Solution. This may dilute the DNA yield in your first elution and it may interfere with the PCR detection, as ethanol is known to be a PCR inhibitor.

10. **What if I forgot to add the Isolation Control (*IsoC*) during the isolation?**
    - It is recommended that the isolation is repeated.

11. **What if I forgot to run the Control PCR for the sample and I only ran the Detection PCR and I obtained a positive result?**
    - The result can be considered positive. However, any negative result must be verified by running the associated control PCR to ensure that it is a true negative and not a false negative due to problems with the DNA isolation or the PCR reactions.

Related Products	Product #
Dirofilaria immitis PCR Detection Kit	44500
Toxoplasma gondii PCR Detection Kit	44700

Technical Assistance

NORGEN's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen's Urine-Based *Trichomonas vaginalis* PCR Detection Kit) or NORGEN products in general, please do not hesitate to contact us.

NORGEN customers are a valuable source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at NORGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362 or call one of the NORGEN local distributors (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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